AD_____

Award Number:
W81XWH-06-1-0090

TITLE:
Amplification of Type II Cadherins in Prostate Cancer

PRINCIPAL INVESTIGATOR:
Teresa L. Johnson-Pais, Ph.D.

CONTRACTING ORGANIZATION:
University of Texas Health Science Center,
San Antonio, TX 78229

REPORT DATE:
November 2009

TYPE OF REPORT:
Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-11-2009	Final	10/30/2005-10/29/2009

4. TITLE AND SUBTITLE
Amplification of Type II Cadherins in Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0090

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Teresa L. Johnson-Pais, Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas Health
Science Center,
San Antonio, TX 78229

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
An Materiel Command
Fort Detrick, MD 21701-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Genomic alterations of 18q have been observed in prostate cancer. This research focused on analyzing the role of increased gene copy number at 18q22.1 found specifically in prostate cancer. We believe the key gene is the cellular adhesion gene cadherin 7 (*CDH7*). The increased copies of *CDH7* results in increased levels of *CDH7* mRNA in tumor cells. We studied the role of loss of expression of *CDH7* on the migratory and invasive potential of prostate cancer cells. We evaluated prostate cancer cells knocked-down for CDH7 expression *in vitro and* found that loss of *CDH7* expression played no role in migratory or invasive potential. We created CDH7 monoclonal antibodies to study protein levels in prostate tumors. These antibodies were tested in immunohistochemistry (IHC) experiments with paraffin-embedde d prostate tissue, but these antibodies were inadequate for IHC. Recently available commercial antibodies were also problematic. Due to the lack of adequate antibody reagents, our future direction will be an evaluation of the copy number of the CDH7 region in prostate tumors by deep sequencing to determine the exact target of the increased copy number at 18q22.1.

15. SUBJECT TERMS
prostate cancer, cadherin, chromosome 18, copy number alteration

16. SECURITY CLASSIFICATION			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
u	u	u	Uu	14	

Table of Contents

Introduction

Genomic alterations at 18q have been observed in prostate cancer. This research focused on analyzing the role of a copy number increase at 18q22.1 in prostate cancer and the potential function of the critical genes that are found to be present in increased copy numbers. This was innovative research in that we are the first to observe increased copy numbers of genes in this region in prostate cancer. We believe the key genes in increased copy are a class of cell adhesion molecules, the type II mesenchymal cadherins. We are studying the role of overexpression of these genes normally expressed in mesenchymal cells, particularly cadherin 7 (*CDH7*), on the invasive potential of prostate cancer epithelial cells. These cadherins have never been implicated in prostate cancer, despite the fact that the CDH7 protein is only expressed in brain, testes and prostate. We analyzed whether loss of expression of *CDH7* played a role in the migratory and/or invasive potential of prostate cancer cells that overexpress *CDH7*.

Body

The research accomplishments for:

Task 1: Identify the smallest common region of amplification at 18q22.1 in prostate cancer.

a. Perform fluorescence in situ hybridization (FISH) on paraffin-embedded prostate tumor specimens using bacterial artificial chromosomes (BACs) spanning the amplified region.

We previously assembled a complete contig of BAC clones that spanned the original region of altered copy number defined by array comparative genomic hybridization (array CGH). Using FISH methodologies on prostate tumors with hybridization probes created by fluorescently-labeling DNA isolated from two BAC clones that flank the *CDH7* gene, we have shown that the smallest region of altered copy number is a 680 kb region, a portion of which is homologous to the chromosome 18 genome found in the BAC clone RPCI11-775G23, which encodes *CDH7* (Figure 1).

a. **b.**

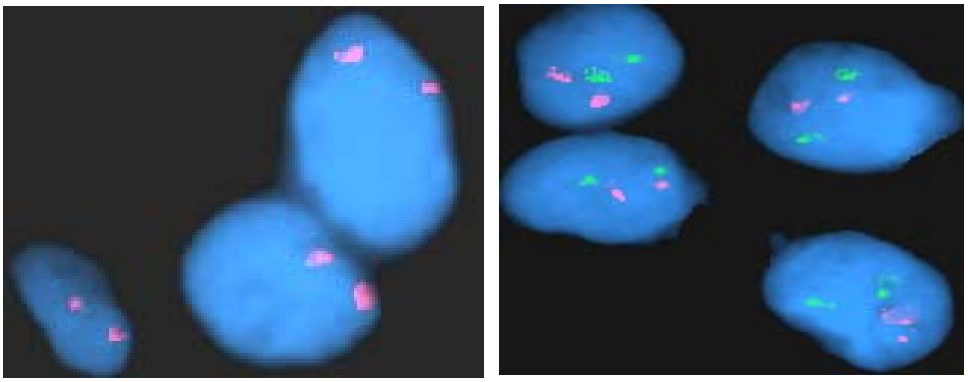

Figure 1: Identification of minimal region of amplification at 18q22.1. A probe proximal to RPCI11-775G23, RPCI11-453M23 (Spectrum Orange) is present in two copies in both tumor (a) and normal prostate tissues (b), while a distal probe RPCI11-425M2 (FITC) is deleted in the prostate tumor section (a). These two probes are present approximately 680 kb apart from each other on the chromosome.

Since the *CDH7* gene is located within this region of increased copy number, we analyzed the copy number of *CDH7* using quantitative PCR. DNA was isolated from microdissected prostate tumors showing increased copy number at 18q22.1 and a quantitative assay to measure *CDH7* gene copy number using real-time PCR was developed. The copy number of the *CDH7* gene in the prostate tumors ranged between two and seven (data not shown). For the majority of samples, the gene copy number of *CDH7,* as detected by quantitative PCR, correlated with the degree of amplification of the region homologous to RP11-775G23 detected by array CGH in the corresponding tumor section.

c. Perform quantitative reverse transcription-PCR on RNA isolated from prostate tumors to verify increased gene expression with increased gene copy number.

We tested RNA extracted from microdissected prostate tumors to verify whether the genomic amplification of the gene has any impact at the transcriptional level of *CDH7*. As expected, we detected three- to eight-fold overexpression of *CDH7* mRNA in prostate tumors compared to the normal adjacent tissue (Figure. 2). The cell line PZ-HPV-7, derived from prostate epithelial cells, showed very low *CDH7* expression. The expression of *CDH7* was several hundred-fold higher in the tumors, compared to PZ-HPV-7 (Fig. 2). Together, array CGH, FISH and real-time quantitative PCR show increased copy number of the *CDH7* gene in prostate cancer which correlates with increased expression of *CDH7*.

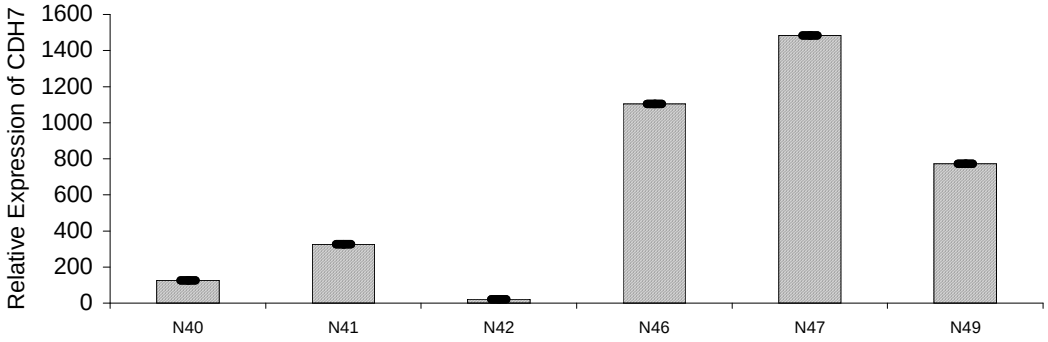

Figure 2: Quantitative real-time RT-PCR analysis of *CDH7* mRNA in prostate tumors. The prostate epithelial cell line PZ-HPV-7 was given a value of 1 for *CDH7* expression and the prostate tumors were shown as fold expression above the level of PZ-HPV-1

Task 1 is completed.

Task 2: Investigate the expression of E-cadherin, N-cadherin, cadherin-7, cadherin-11, cadherin-19 and cadherin-20 proteins in prostate tumors of varying stages and grades.

We wanted to investigate the expression of E-cadherin, N-cadherin and cadherin-11 in prostate tumors because E-cadherin has been found to be down-regulated in prostate tumors (Rubin et al., 2001) and co-expression of two mesenchymal cadherins N-cadherin and cadherin-11 have been reported in prostate cancer samples (Tomita et al., 2000). *CDH7* is within our minimal region of increased copy number and the cadherin-19 gene is approximately 700 kb distal to the *CDH7.* The gene for another mesenchymal cadherin, cadherin-20 is located 5 megabases

proximal to *CDH7* and does not appear to be in increased copy number. This study would give a more complete picture of changes in cadherin expression during prostate cancer progression.

a. Create antibodies to specifically recognize the type II cadherins (cadherin-7 and -19).

As reported in our first annual report (November 2006), we contracted with Sigma-Genosys to create two custom polyclonal antibodies against CDH7 and CDH19 using unique peptides from the extracellular domain and at the COOH-terminus. We received the antisera and performed some preliminary characterization of the antibodies. Our initial results showed that the antibodies directed against the extracellular domain peptides appeared to recognize the appropriate size of protein from cell lysates, as detected by western blotting. However, prior to their use in immunohistochemistry experiments, the antisera needed further purification. We attempted many types of purification strategies, and a collaborator had success with the antibodies on paraffin-embedded brain tissue, but the antibodies directed against the small peptides were inadequate for immunohistochemical analysis of paraffin-embedded prostate tissue.

Since the peptide antibodies we previously created were not suitable for our purposes, we created mouse monoclonal antibodies directed against bacterially-expressed full-length CDH7 and CDH19 with the help of the UTHSCSA Institutional Antibody core laboratory. We assessed the purified hybridoma supernatants containing the monoclonal antibodies against human CDH7 and CDH19. Table 1 summarizes our screening results.

Table 1: Summary of anti-CDH7 and anti-CDH19 hybridoma supernatants acceptable for use for western blotting[a] and immunohistochemistry (IHC)[b] experiments.

Anti-CDH7 monoclonal antibodies	Western Blot	IHC	Anti-CDH19 Monoclonal antibodies	Western Blot	IHC
8E2	No	No	4D1	No	No
17G1	Yes	Yes	7D6	No	No
23E5	No	No	16B7	No	No
21F2	No	No	19A8	No	No
25C8	No	No	20BII	No	No
IF8	Yes	Yes	18H7	Yes	No
IID9	No	No	22GII	No	No
IIG8	No	No	5HIIA	Yes	No
8H6	No	No	20CIIB	No	Yes
22GII	No	No	15C1A	No	No
9A9	No	Yes	11D9	No	Yes
162	No	No	1DE6	Yes	No
4D3	No	No	15C1B	No	No
5DI	No	Yes	5HIIB	No	No
5D9	No	No	20CIIA	Yes	Yes
-----	-----		23E5	No	No

[a]Refers to bacterially-expressed fusion protein lysate used for western blot
[b] Refers to 22RV1 prostate cells used for IHC

Figure 3 demonstrates the results we obtained from one immunohistochemistry experiment performed using the 22Rv1 human prostate cancer cell line that contains increased copy number of the chromosome 18 region containing the *CDH7* gene and the anti-CDH7 monoclonal antibody 9A9 as the primary antibody. However, use of the antibody in immunohistochemistry experiments using paraffin-embedded tissue proved these antibodies were inadequate for use with paraffin-embedded tissue. In addition, two commercial suppliers of antibodies (Santa Cruz Biotechnology and AbCam) have developed rabbit polyclonal antibodies against CDH7 protein and use of these antibodies in immunohistochemistry experiments also proved problematic. Since our prostate cancer tissue biorepository consists of primarily paraffin-embedded tissues and our prostate tumor tissue microarray consisted of paraffin-embedded tissues, we were unable to assess the expression level of CDH7 protein in prostate tumors with or without increased copy number of *CDH7*.

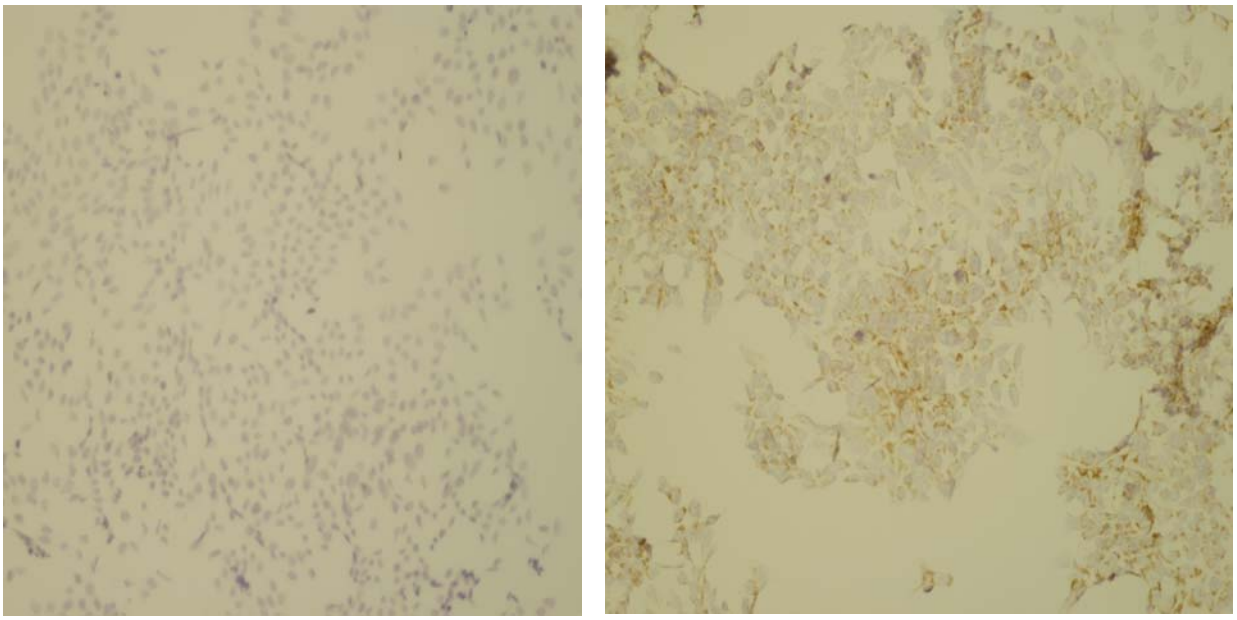

Figure 3: IHC performed using 22RV1 human prostate cancer cells. Left panel, negative control. Right panel, 22RV1 cells incubated with a 1:100 dilution of the anti-CDH7 9A9 hybridoma supernatant

In response to the Reviewer: In trying to create antibodies directed against CDH7 and CDH19, we used all of the resources that were available to us. We tried a commercial source of polyclonal rabbit antibodies using a small polypeptide antigen. We tried a core laboratory using GST- and MBP-labeled domains of the proteins to create both mouse monoclonal and rabbit polyclonal antibodies and we used some recently available commercial antibodies. We were just as disappointed as the reviewer that none of these antibodies would adequately work on the paraffin-embedded prostate tissue. One of the problematic issues for this task is the fact that the cadherin family members are highly-related. The development of specific antibodies for a single member of a highly-related family is not always an easy task. Prior to the submission of this proposal, we spoke with several protein/antibody experts, and because of several amino acid differences in the C-terminal regions of the different cadherin family members, they believed that we would be successful in our attempt to create anti-CDH7 and anti-CDH19

specific antibodies. However, ultimately this is still research and the end results are not always what are desired.

b. Prepare tissue microarrays using prostate cancer specimens of various stages and grades.

Imgenex (San Diego, CA) is a commercial supplier of tissue microarrays and they have developed a prostate tumor tissue microarray consisting of 40 tumors or various stages and Gleason grades with matched normal tissue. FISH was performed on this microarray using a hybridization probe derived from the BAC clone RPCI11-775G23 that contains the *CDH7* gene. All 40 prostate tumors showed three or more signals from the RPCI11-775G23 probe (data not shown). The control probe, a centromeric probe from chromosome 18, showed one to two signals in all 40 tumors on the array indicating that chromosome 18 was not completely amplified in the tumors. These results were consistent with our original array CGH data which showed no correlation between *CDH7* copy number and Gleason score in prostate tumors from the UTHSCSA prostate tumor biorepository (Table 2). This led us to hypothesize that the mechanism that results in increased copies of *CDH7* is an early event during tumorigenesis and may be a predisposing factor to the development of prostate cancer.

Table 2. Prostate tumor samples with various pathological grades showing amplification at chromosome 18q22.1

Tumor	Ag	Gleason	Stage	Amplification at 18q22.1
N10	66	6	T2CNXMX	Medium
N12	64	6	T2CNXMX	Mediu
N15	72	7	T2CNXMX	Mediu
N22	59	7	T2CN0M	High
N26	56	6	T2CNXMX	Lo
N30	73	5	T2CNXMX	Mediu
N31	72	6	T2CNXMX	Mediu
N32	54	8	T2CN0M	Lo
N34	52	7	T3BN0M	Norma
N35	56	7	T2CNXMX	Lo
N36	62	6	T2CNXMX	Mediu
N37	62	9	T3AN0M	High
N38	59	6	T2CNXMX	Mediu
N39	60	7	T3NXMX	Lo
N40	70	7	T2CNXMX	High
N41	67	7	T3BN0M	High
N42	67	8	T2CN0M	Lo
N43	65	5	T2BNXMX	Lo
N44	53	7	T2BNXMX	Mediu
N45	55	9	T2BN0M	Lo
N47	71	9	T3BN0M	Lo
N49	56	9	T2CNXMX	Lo

* $_2$ ratio 1-1.5: Low; Log $_2$ ratio 1.5-2.0: Medium; Log $_2$ ratio >2.0: High

c. Perform immunohistochemistry experiments to analyze expression of E-cadherin, N-cadherin and the type II cadherins, cadherin-7, -11, -19 and -20 using tissue microarrays.

Since we encountered difficulty with our development of antibodies against CDH7 and CDH19 that would be adequate for immunohistochemistry experiments, we were not able to proceed with this task. We were frustrated that we were unable to develop or purchase antibody reagents that would provide a more complete picture of the cadherin expression changes occurring during the tumorigenic process in prostate cells.

d. Perform experiments to test the tissue specificity of increased copy number of the three type II cadherins (cadherin-7, -19 and –20).

We performed FISH experiments with the RPCI11-775G23 (contains *CDH7*) probe on tissue microarrays consisting of cancer from 12 organ sites (Imgenex common cancers A and B) The gene copy number alteration detected on chromosome 18q22.1 using probe RPCI11-775G23 on prostate tumor samples was not observed in any other tumors samples, including stomach, esophagus, lung, colon, thyroid, kidney, breast, liver, urinary bladder, ovary and pancreas. These data indicate that the increased copy number of the RPCI11-775G23 region containing *CDH7* is tumor specific, and is limited to the prostate. Of these 12 tissues analyzed, only prostate is known to express *CDH7* (Kools et al., 2000). Since *CDH7* is also expressed in testes (Kools et al., 2000), we have analyzed a small number of testicular tumors by FISH and have shown that *CDH7* is also in increased copy number.

Tasks 2b and d w ere completed. We were unable to complete Tasks 2a and 2c due to a lack of adequate antibody reagents.

Task 3: Knockdown expression of type II cadherins in prostate cancer cell lines and analyze the phenotype of the cells for invasive and tumorigenic potential.

a. Create transient and stable RNA interference constructs and perform experiments to knockdown expression of cadherin-7, -19 or -20, individually.

We designed short hairpin RNAs (shRNA) for cadherin-7, -19 and -20 using design tools provided by Ambion (The Woodlands, TX). The shRNA was cloned into the pSilencer vector (Ambion) which contains a mammalian selectable marker for creating RNA interference constructs that can be stably selected in a mammalian cell line. The shRNA construct for *CDH7* was stably transfected into the 22Rv1 human prostate cancer cell line, a cell line that expresses *CDH7*. Analysis of the *CDH7* mRNA in the stable transfectants showed dramatic reduction in the mRNA levels.

b. Analyze the transformed metastatic phenotype of the prostate cancer cells after knockdown of the type II cadherins using *in vitro* assays for migratory and invasive potential.

We evaluated two *in vitro* assays from Calbiochem (Innocyte; Gibbstown, NJ) for determining the migratory and invasive potential of the parental 22Rv1 prostate cancer cell line. These prostate cancer cells show increased copy number of *CDH7* by FISH and elevated mRNA levels

for *CDH7* compared to normal prostate cells. The Innocyte migration assay is an assay for cell migration in 24-well plates. Cells migrate through an 8 μm pore to a feeder layer containing serum as a chemoattractant. Migrated cells are quantitated using a cell-permeable fluorescent dye. The Innocyte invasion assay quantitatively measures cell invasion. The assay contains cell culture inserts with an 8 μm polycarbonate membrane. This membrane is coated with a thin layer of a biological matrix. This layer prevents noninvasive cells from going through the membrane. Cells are quantified utilizing a highly sensitive fluorescent dye. Preliminary results from the migration assay using 22Rv1 cells demonstrated that there was a 10-fold increase in cell migration when media containing 10% serum was used as a chemoattractant compared to serum free media (data not shown). In the invasion assay, 22Rv1 cells did not show invasive potential using serum as a chemoattractant (data not shown). We tested the 22Rv1 cells knocked-down for expression of *CDH7* in the migration and invasion assays. Analysis of these assays showed that loss of expression of *CDH7* did not affect the migratory or invasive potential of the prostate cancer cells compared to the parental control cells (data not shown).

c. Analy ze the *in vivo* tumorigenic phenotype of the prostate cancer cells after knockdown of type II cadherin mRNA.

Due to the lack of experimental evidence that loss of expression of *CDH7* would contribute to a change in cellular migration and/or invasion, the *CDH7* knocked-down prostate cancer cells were not introduced into a mouse model to analyze *in vivo* tumorigenic potential.

In response to the Reviewer: We lacked antibody reagents to verify that the amount of CDH7 protein was indeed altered in prostate cancer tissue. We also observed that the knockdown of CDH7 in prostate cancer cells did not cause a change in the in vitro cancerous phenotype of the 22RV-1 prostate cancer cells. These results did not give us confidence that CDH7 was indeed the target of the increased copy number on chromosome 18. Mouse experiments are costly and without preliminary data to show that the experiment would provide us with meaningful data we did not proceed with this part of the task. We did not test the knockdown of CDH19 or 20 in vivo for changes in tumorigenic phenotype, because these cadherins are not located in the minimal region of copy number gain and would have served only as controls. With funding from another source, we are in the process of analyzing this region through deep sequencing to verify that CDH7 is the target of the copy number gain and then we will have preliminary data to justify the mouse costs of testing the CDH7 knockdown prostate cancer cells in vivo.

Task 3a and b were completed.

Key Research Accomplishments

- **Defined a minimal region of increased copy number (680kb) of 18q22.1 containing the cadherin 7 gene in prostate cancer.**
- **Determined that increased copy number of the cadherin-7 gene results in increased cadherin-7 mRNA levels in prostate tumors**
- **Determined that increased copy number of the cadherin-7 gene does not correlate with the stage or Gleason score of prostate tumors.**
- **Determined that increased copy number of the cadherin-7 gene is found specifically in prostate cancer and not 11 other common cancers.**
- **Knocked down expression of the cadherin-7 gene in the prostate cancer cell line 22Rv1 that contains increased copy number of the cadherin-7 gene.**

- **Evaluated migratory and invasive potential of 22Rv1 cells knocked-down for *CDH7* expression.**

Reportable Outcomes

We previously submitted our work as a manuscript to the scientific journals Genes, Chromosomes and Cancer and Oncogene, however without antibody resources to provide data demonstrating the amount of CDH7 protein in the prostate tumors our manuscript has not been judged acceptable for publication (included in the appendix in first annual report – November 2006). Our results have been presented as a poster at the DOD Innovative Minds in Prostate Cancer Today meeting held in Atlanta, GA in September 2007 and the International Congress of Human Genetics held in Brisbane, Australia in August 2006.

Conclusions

We demonstrated that the region of increased copy number in prostate cancer at 18q22.1, originally detected by array CGH, can be minimized to a 680 Kb region that contains the cadherin-7 gene. This increased copy number of the cadherin-7 gene is specific to prostate cancer and is not found in 11 other common cancers. The increased copy number of the cadherin-7 gene also results in increased levels of cadherin-7 mRNA in prostate tumors compared to the adjacent normal prostate tissue. We have performed knockdown experiments of the cadherin-7 mRNA in a prostate cancer cell line and used *in vitro* assays to determine that prostate cancer cells lines with reduced cadherin-7 protein expression do not exhibit altered migratory or invasive potential.

We have not found that increased number of the cadherin-7 gene is correlated with the stage or Gleason score of the prostate tumors and may be an early marker of prostate cancer. Since the cadherins are a class of cell adhesion molecules and the type II cadherins are mesenchymal cadherins not normally expressed in epithelial cells, the increased expression of cadherin-7 may be a marker of the tumorigenic phenotype. However, it is possible that the target of the increased copy number of this region is not only cadherin-7. The lack of a change in migratory and invasive potential of prostate cancer cells after expression of *CDH7* is reduced is one piece of evidence that CDH7 might not be the only gene whose altered gene dosage plays a role in prostate cancer. The future directions for this project is the deep sequencing of this region in prostate tumors to more precisely pinpoint the target of the increased copy number.

References

Kools P, Van Imschoot G, van Roy F. 2000. Characterization of three novel human cadherin genes (*CDH7*, CDH19, and CDH20) clustered on chromosome 18q22-q23 and with high homology to chicken cadherin-7. Genomics 68:283-295.

Rubin MA, Mucci NR, Figurski J, Fecko A, Pienta KJ, Day ML (2001) E-cadherin expression in prostate cancer: a broad survey using high-density tissue microarray technology. Human Path 32:690-697.

Tomita K, van Bokhoven A, van Leenders GJLH, Ruijter ETG, Jansen CFJ, Bussemakers MJG, Schalken JA (2000) Cadherin switching in human prostate cancer progression. Cancer Res 60:3650-3654.

Appendix

DOD IMPaCT meeting abstract, Atlanta, GA (2007)

Title: GAIN OF COPY NUMBER OF AN 18q22.1 REGION THAT INCLUDES THE CADHERIN-7 GENE IN PROSTATE CANCER

Author(s):
Teresa L. Johnson-Pais; Veronica E. Contreras-Shannon; Sapna Vijayakumar (Mount Sinai School of Medicine, New York, New York); Susan L. Naylor and Robin J. Leach.

Presenter: Teresa L. Johnson-Pais

Abstract:
Genomic alterations of the 18q chromosomal region have been observed in prostate cancer. We performed array comparative genomic hybridization experiments using prostate cancer specimens and identified the gain of copy number of a small region at 18q22.1. This relatively gene-poor region includes two cellular adhesion genes, the type II cadherins cadherin 7 and cadherin 19. Alteration of expression of another type II cadherin, cadherin 11, is implicated in tumor invasiveness and metastasis in both breast and prostate cancer. Therefore, we sought to study if the increased copy number of the 18q22.1 region in prostate cancer results in upregulation of expression of the type II cadherins, cadherin 7 and/or cadherin 19, which may play a role in prostate cancer development and/or progression. We verified the gain of copy number of this region using fluorescence in situ hybridization (FISH) and determined the copy number to range from 3-7 copies. The smallest region of copy number alteration was identified by performing FISH on paraffin-embedded prostate tumors using probes derived from bacterial artificial chromosomes containing 18q22.1 genomic sequences. This narrowed region was 680 kilobases and included the cadherin 7 gene and no other known genes. Additionally, FISH analyses of 12 other tumor types showed that the gain of copy number at 18q22.1 was specific to prostate tumor samples. A survey of 40 prostate tumors with various Gleason scores and stages showed that the gain of copy number of this region was not associated with a particular stage or grade. Polyclonal rabbit antibodies generated against small peptides from cadherins 7 and 19 were created for use in immunohistochemistry experiments to analyze the level of protein associated with extra copies of the gene. Extensive testing of these antibodies revealed they were inadequate for immunohistochemistry. Therefore, new antibodies are being generated against bacterially expressed cadherin 7 and cadherin 19 fusion proteins. We have knocked down RNA expression of cadherin 7 using RNA interference in the 22Rv1 prostate cancer cell line. Following the generation of antibodies to detect protein expression in the knocked-down 22Rv1 cells, *in vitro* assays for cellular invasiveness and *in vivo* assays for tumorigenicity will be performed. IMPACT: The discovery of increased copy number of a cellular adhesion gene in prostate cancer could lead to a better understanding of how normal cellular adhesion is altered during acquisition of the metastatic phenotype. Understanding these events could potentially lead to the development of new therapeutics designed to interfere with the metastatic process.

International Congress of Human Genetics Meeting abstract, Brisbane, Australia (2006)

Title: Prostate Cancer is Associated with Cadherin-7 Gene Amplification

Authors: Verónica Contreras-Shannon, Sapna Vijayakumar, Xavier T. Reveles, Devon C. Hall, Dean A. Troyer, Susan L. Naylor, Robin J. Leach and Teresa L. Johnson-Pais.

The incidence of prostate cancer continues to rise. One in six men is diagnosed with prostate cancer, which accounts for 30,000 deaths per year in the U.S. Still, prostate cancer diagnosis has not improved appreciably in the past decade. Prostate-specific antigen (PSA) is used for the early detection of prostate cancer, however, a prevalence of prostate cancer was recently reported in men with “normal” PSA levels—emphasizing the importance of identifying sensitive biomarkers for detecting prostate cancer. The purpose of this study was to identify regions of loss and gain in prostate cancer tissue on chromosome 18q, a chromosome which suppresses prostate cancer tumorigenicity and metastases. A copy number amplification of chromosome 18q22.1, containing the gene for cadherin-7 (*CDH7*), was observed in several primary prostate tumors. Amplification of *CDH7* was confirmed by FISH and real-time quantitative PCR. By FISH, three or more copies of *CDH7* were observed in 60 out of 60 prostate tumors, as well as testicular cancer, but not adjacent/control tissues or other cancers. All prostate tumor stages and grades showed amplification of *CDH7*. Because cadherin-7 may be associated with changes in cell adhesion related to invasion and metastases of cancer cells, we initiated an examination of the functional role of this protein in cancer specimens and a prostate cancer cell-line, 22RV1. First, an antibody specific to cadherin-7 was generated and used to screen and score normal and prostate cancer specimens. Stable RNA interference will be used to knockdown expression of cadherin-7 and analyze the transformed/metastatic phenotype of prostate cancer cells using *in vitro* assays for anchorage independence and migration/invasion. Based on these studies, cadherin-7 may be an important and novel biomarker. The amplification of *CDH7* at all stages of prostate cancer suggests this is an early event and, therefore, a promising marker for the early detection of prostate cancer.

Personnel supported at various times by this funding

Teresa L. Johnson-Pais, Ph.D	Principal Investigator
Devon C. Hall	Graduate Student
Veronica Contreras-Shannon, Ph.D.	Postdoctoral Fellow
Emily Rhodes, Ph.D.	Postdoctoral Fellow
Mandy Rolando	Research Associate
Jen Franke	Research Assistant
Stephanie DeMoor	Undergraduate summer student